

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

WEST[Generate Collection](#)[Print](#)

Search Results - Record(s) 1 through 23 of 23 returned.

☐ 1. Document ID: US 20030100467 A1

L5: Entry 1 of 23

File: PGPB

May 29, 2003

PGPUB-DOCUMENT-NUMBER: 20030100467

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030100467 A1

TITLE: Binding phenol oxidizing enzyme-peptide complexes

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Aehle, Wolfgang	Delfgauw	CA	NL	
Baldwin, Toby M.	Palo Alto	CA	US	
van Gastel, Franciscus J. C.	Union City	CA	US	
Janssen, Giselle G.	San Carlos	CA	US	
Murray, Christopher J.	Soquel	CA	US	
Wang, Huaming	Fremont	CA	US	
Winetzky, Deborah S.	Foster City		US	

US-CL-CURRENT: 510/392; 435/183, 510/530

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	EMOC
Draw	Desc	Image									

☐ 2. Document ID: US 20030054369 A1

L5: Entry 2 of 23

File: PGPB

Mar 20, 2003

PGPUB-DOCUMENT-NUMBER: 20030054369

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054369 A1

TITLE: Method for detection of Stachybotrys chartarum in pure culture and field samples using quantitative polymerase chain reaction

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cruz-Perez, Patricia	Las Vegas	NV	US	
Buttner, Mark P.	Henderson	NV	US	

US-CL-CURRENT: 435/6; 435/254.1, 435/91.2, 536/24.3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC
Draw Desc	Image										

☐ 3. Document ID: US 20020165113 A1

L5: Entry 3 of 23

File: PGPB

Nov 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020165113

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020165113 A1

TITLE: Detergent compositions comprising novel phenol oxidizing enzymes

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Aehle, Wolfgang	Delfgauw	CA	NL	
Convents, Daniel	Vlaardingen	CA	NL	
Doornink, Monique	Vlaardingen	CA	NL	
Van Gastel, Franciscus	Union City	CA	US	
Rodriguez, Ana Milena	Mountain View		US	
Toppozada, Amr	San Francisco		US	
De Vries, Cornelis Hendrikus	Vlaardingen		NL	
Wang, Huaming	Fremont		US	

US-CL-CURRENT: 510/392

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC
Draw Desc	Image										

☐ 4. Document ID: US 20020160389 A1

L5: Entry 4 of 23

File: PGPB

Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020160389

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160389 A1

TITLE: Method for generating a library of mutant oligonucleotides using the linear cyclic amplification reaction

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rodriguez, Ana M.	Mundelein	IL	US	
Wang, Huaming	Fremont	CA	US	

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/69.1, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 5. Document ID: US 20020155439 A1

L5: Entry 5 of 23

File: PGPB

Oct 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020155439

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155439 A1

TITLE: Method for generating a library of mutant oligonucleotides using the linear cyclic amplification reaction

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rodriguez, Ana	Mundelein	IL	US	
Schellenberger, Volker	Palo Alto	CA	US	
Wang, Huaming	Fremont	CA	US	

US-CL-CURRENT: 435/6; 435/69.1, 435/7.1, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 6. Document ID: US 20020151450 A1

L5: Entry 6 of 23

File: PGPB

Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020151450

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020151450 A1

TITLE: Novel phenol oxidizing enzymes

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wang, Huaming	Fremont	CA	US	

US-CL-CURRENT: 510/320; 435/189, 435/252.3, 435/254.1, 435/320.1,
435/69.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw	Desc	Image								

☐ 7. Document ID: US 20020142423 A1

L5: Entry 7 of 23

File: PGPB

Oct 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020142423
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020142423 A1

TITLE: Phenol oxidizing enzymes

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wang, Huaming	Fremont	CA	US	
Bodie, Elizabeth A.	San Carlos	CA	US	

US-CL-CURRENT: 435/189; 435/254.2, 435/320.1, 435/69.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw	Desc	Image								

☐ 8. Document ID: US 20020081642 A1

L5: Entry 8 of 23

File: PGPB

Jun 27, 2002

PGPUB-DOCUMENT-NUMBER: 20020081642
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020081642 A1

TITLE: Method for detecting antibodies to and antigens of fungal
and yeast exposures

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cherwonogrodzky, John W.	Medicine Hat	CA		

US-CL-CURRENT: 435/7.31; 424/274.1, 435/254.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KMIC

☐ 9. Document ID: US 20020039772 A1

L5: Entry 9 of 23

File: PGPB

Apr 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020039772

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020039772 A1

TITLE: Method of increasing recovery of heterologous active enzymes produced in plants

PUBLICATION-DATE: April 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hood, Elizabeth	College Station	TX	US	
Howard, John A.	College Station	TX	US	
Bailey, Michele	College Station	TX	US	
van Gastel, Franciscus J.C.	Union City	CA	US	
Ward, Michael	San Francisco	CA	US	
Wang, Huaming	Fremont	CA	US	
Woodard, Susan	College Station	TX	US	

US-CL-CURRENT: 435/183

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KMIC

☐ 10. Document ID: US 20020019038 A1

L5: Entry 10 of 23

File: PGPB

Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019038

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020019038 A1

TITLE: PHENOL OXIDIZING ENXYMES

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
HUAMING, WANG	FREMONT	CA	US	

US-CL-CURRENT: 435/189

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KWC

☐ 11. Document ID: US 20020015957 A1

L5: Entry 11 of 23

File: PGPB

Feb 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020015957

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020015957 A1

TITLE: Diagnostics and therapeutics for macular
degeneration-related disorders

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hageman, Gregory S.	Coralville	IA	US	
Mullins, Robert F.	Coralville	IA	US	

US-CL-CURRENT: 435/6; 351/200

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KWC

☐ 12. Document ID: US 6509307 B1

L5: Entry 12 of 23

File: USPT

Jan 21, 2003

US-PAT-NO: 6509307

DOCUMENT-IDENTIFIER: US 6509307 B1

TITLE: Detergent compositions comprising phenol oxidizing enzymes
from fungi

DATE-ISSUED: January 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bodie; Elizabeth Ann	San Carlos	CA		
van der Velden; Sebastiaan	Vlaardingen			NL
de Vries; Comelis Hendrikus	Vlaardingen			NL
Wang; Huaming	Fremont	CA		

US-CL-CURRENT: 510/226; 435/189, 435/911, 530/350, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KMC

☐ 13. Document ID: US 6426410 B1

L5: Entry 13 of 23

File: USPT

Jul 30, 2002

US-PAT-NO: 6426410

DOCUMENT-IDENTIFIER: US 6426410 B1

TITLE: Phenol oxidizing enzymes

DATE-ISSUED: July 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Huaming	Fremont	CA		

US-CL-CURRENT: 536/23.2; 435/252.3, 435/252.31, 435/252.33,
435/254.11, 435/254.3, 435/320.1, 435/471, 435/484, 435/485 ,
435/488, 435/69.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KMC

☐ 14. Document ID: US 6399329 B1

L5: Entry 14 of 23

File: USPT

Jun 4, 2002

US-PAT-NO: 6399329

DOCUMENT-IDENTIFIER: US 6399329 B1

TITLE: Phenol oxidizing enzymes

DATE-ISSUED: June 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Huaming	Fremont	CA		
Bodie; Elizabeth A.	San Carlos	CA		

US-CL-CURRENT: 435/69.1; 435/189, 435/252.3, 435/254.11, 435/254.2,
536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw Desc	Image								

KMC

☐ 15. Document ID: US 6387652 B1

L5: Entry 15 of 23

File: USPT

May 14, 2002

US-PAT-NO: 6387652

DOCUMENT-IDENTIFIER: US 6387652 B1

TITLE: Method of identifying and quantifying specific fungi and bacteria

DATE-ISSUED: May 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Haugland; Richard	Middleton	OH		
Vesper; Stephen	Kettering	OH		

US-CL-CURRENT: 435/34; 435/254.1, 435/254.3, 435/254.5, 435/254.6,
435/4, 435/6, 435/848, 435/849, 435/913, 435/968, 435/973

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 16. Document ID: US 6168936 B1

L5: Entry 16 of 23

File: USPT

Jan 2, 2001

US-PAT-NO: 6168936

DOCUMENT-IDENTIFIER: US 6168936 B1

TITLE: Phenol oxidizing enzymes

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Huaming	Fremont	CA		

US-CL-CURRENT: 435/189; 435/320.1, 435/69.1, 536/23.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 17. Document ID: US 6058940 A

L5: Entry 17 of 23

File: USPT

May 9, 2000

US-PAT-NO: 6058940

DOCUMENT-IDENTIFIER: US 6058940 A

TITLE: Method and system for assay and removal of harmful toxins during processing of tobacco products

DATE-ISSUED: May 9, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lane; Kerry Scott	Del Ray Beach	FL	33483	

US-CL-CURRENT: 131/298; 131/297, 131/300, 131/309, 131/310

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 18. Document ID: US 6017751 A

L5: Entry 18 of 23

File: USPT

Jan 25, 2000

US-PAT-NO: 6017751

DOCUMENT-IDENTIFIER: US 6017751 A

TITLE: Process and composition for desizing cellulosic fabric with an enzyme hybrid

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
von der Osten; Claus	Lyngby			DK
Bjornvad; Mads E.	Frederiksberg			DK
Vind; Jesper	Lyngby			DK
Rasmussen; Michael Dolberg	Vallensbaek			DK

US-CL-CURRENT: 435/263; 435/198, 435/202, 435/69.7, 435/71.1, 510/530

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 19. Document ID: US 6015783 A

L5: Entry 19 of 23

File: USPT

Jan 18, 2000

US-PAT-NO: 6015783

DOCUMENT-IDENTIFIER: US 6015783 A

TITLE: Process for removal or bleaching of soiling or stains from cellulosic fabric

DATE-ISSUED: January 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP	CODE	COUNTRY
von der Osten; Claus	Lyngby				DK
Cherry; Joel R.	Davis	CA			
Bjornvad; Mads E.	Frederiksberg				DK
Vind; Jesper	Lyngby				DK
Rasmussen; Michael Dolberg	Vallensbaek				DK

US-CL-CURRENT: 510/392

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 20. Document ID: US 5916798 A

L5: Entry 20 of 23

File: USPT

Jun 29, 1999

US-PAT-NO: 5916798

DOCUMENT-IDENTIFIER: US 5916798 A

TITLE: Method of obtaining a cellulosic textile fabric with reduced tendency to pilling formation

DATE-ISSUED: June 29, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP	CODE	COUNTRY
Lund; Henrik	Copenhagen				DK
Pedersen; Hanne H.o slashed.st	Lyngby				DK

US-CL-CURRENT: 435/263

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 21. Document ID: US 5516674 A

L5: Entry 21 of 23

File: USPT

May 14, 1996

US-PAT-NO: 5516674

DOCUMENT-IDENTIFIER: US 5516674 A

TITLE: Insecticide resistance associated cytochrome 450

DATE-ISSUED: May 14, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roe; Richard M.	Cary	NC		
Hodgson; Ernest	Raleigh	NC		
Rose; Randy L.	Clayton	NC		

US-CL-CURRENT: 435/189; 435/252.3, 435/252.31, 435/252.34,
435/320.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 22. Document ID: WO 2079513 A2

L5: Entry 22 of 23

File: EPAB

Oct 10, 2002

PUB-NO: WO002079513A2

DOCUMENT-IDENTIFIER: WO 2079513 A2

TITLE: METHOD FOR DETECTION OF *<i>STACHYBOTRYS CHARTARUM</i>* IN
PURE CULTURE AND FIELD SAMPLES USING QUANTITATIVE POLYMERASE CHAIN
REACTION

PUBN-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME

COUNTRY

CRUZ-PEREZ, PATRICIA

BUTTNER, MARK P

ASSIGNEE-INFORMATION:

NAME

COUNTRY

UNIV NEVADA LAS VEGAS

US

APPL-NO: US00206335

APPL-DATE: February 28, 2002

PRIORITY-DATA: US28071201P (March 29, 2001)

INT-CL (IPC): C12 Q 1/68

EUR-CL (EPC): C12Q001/68

ABSTRACT:

A method for detecting the fungus Stachybotrys chartarum includes isolating DNA from a sample suspected of containing the fungus Stachybotrys chartarum. The method further includes subjecting the DNA to polymerase chain reaction amplification utilizing at least one of several primers, the several primers each including one of the base sequences 5'GTTGCTTCGGCGGGAAC3', 5'TTTGCGTTTGCCACTCAGAG3', 5'ACCTATCGTTGCTTCGGCG3', and 5'GCGTTTGCCACTCAGAGAATACT3'. The method additionally includes detecting the fungus Stachybotrys chartarum by visualizing the product of the polymerase chain

. reaction.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KWIC
Draw Desc	Image									

☐ 23. Document ID: US 20030054369 A1 WO 200279513 A2

L5: Entry 23 of 23

File: DWPI

Mar 20, 2003

DERWENT-ACC-NO: 2003-148258

DERWENT-WEEK: 200323

COPYRIGHT 2003 DERWENT INFORMATION LTD

TITLE: Detecting and quantifying fungus Stachybotrys chartarum comprises isolating DNA from sample, subjecting DNA to polymerase chain reaction amplification using primer, and detecting fungus by visualizing amplified product

INVENTOR: BUTTNER, M P; CRUZ-PEREZ, P

PATENT-ASSIGNEE:

ASSIGNEE

CODE

BUTTNER M P

BUTTI

CRUZ-PEREZ P

CRUZI

UNIV NEVADA LAS VEGAS

UYNEN

PRIORITY-DATA: 2001US-280712P (March 29, 2001), 2002US-0080959
(February 22, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20030054369 A1	March 20, 2003		000	C12Q001/68
WO 200279513 A2	October 10, 2002	E	040	C12Q001/68

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN
CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN
IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ
NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG
UZ VN YU ZA ZM ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT
KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20030054369A1	March 29, 2001	2001US-280712P	Provisional
US20030054369A1	February 22, 2002	2002US-0080959	
WO 200279513A2	February 28, 2002	2002WO-US06335	

INT-CL (IPC): C07 H 21/04; C12 N 1/16; C12 P 19/34; C12 Q 1/68

ABSTRACTED-PUB-NO: WO 200279513A

BASIC-ABSTRACT:

NOVELTY - Detecting and quantifying the presence of fungus Stachybotrys chartarum, comprises isolating DNA from a sample suspected of containing the fungus *S. chartarum*, subjecting the DNA to polymerase chain reaction (PCR) amplification utilizing at least one primer base sequence, and detecting the fungus *S. chartarum* by visualizing the product of the PCR.

DETAILED DESCRIPTION - Detecting and quantifying the presence of fungus Stachybotrys chartarum, comprises isolating DNA from a sample suspected of containing the fungus *S. chartarum*, subjecting the DNA to polymerase chain reaction (PCR) amplification utilizing at least one primer base sequence which comprises at least one of the sequences (S1) 5'-GTTGCTTCGGCGGAAC-3', and (S2) 5'-TTTGCCTTTGCCACTCAGAG-3', or (S3) 5'-ACCTATCGTTGCTTCGGCG-3', and (S4) 5'-GCGTTTGCCACTCAGAGAATACT-3'. Also a probe that is specific for the fungal species *S. chartarum*, collecting the sample from the environment, extracting the sample's DNA, obtaining DNA standards from a culture of *S. chartarum*, determining the concentration of *S. chartarum* spores in the DNA standards, amplifying by PCR each of the DNA standards and the collected sample's DNA using the obtained primer set and probe, and comparing amplification plots obtained by PCR of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *S. chartarum* in the collected sample and a concentration of the fungus *S. chartarum* in the collected sample, or detecting the fungus *S. chartarum* by visualizing the product of the PCR.

INDEPENDENT CLAIMS are included for;

(1) A primer set (I) for detecting *S. chartarum* using PCR, comprises a first primer comprising S1 and a second primer comprising S2, or a first primer comprising S3 and a second primer comprising S4; and

(2) A primer and probe set for detecting *S. chartarum* using PCR, comprises (I) and probe comprising a base sequence 6-FAM-5'-CTGCGCCCGGATCCAGGC-3'-T- AMRA.

USE - The method is useful for detecting and quantifying the fungus Stachybotrys chartarum in a sample (claimed).

ADVANTAGE - The method detects and absolutely quantifies *S. chartarum* without the necessity of further employing estimated quantization techniques and thus avoids inaccuracies of estimated quantization, where PCR inhibitors may co-extract with the DNA.

CHOSEN-DRAWING: Dwg.0/8

TITLE-TERMS: DETECT QUANTIFICATION FUNGUS STACHYBOTRYS COMPRISE ISOLATE DNA SAMPLE SUBJECT DNA POLYMERASE CHAIN REACT AMPLIFY PRIME DETECT FUNGUS AMPLIFY PRODUCT

DERWENT-CLASS: B04 C07 D13 D16 J04

CPI-CODES: B04-E03; B04-E05; B04-F09; B11-C08E3; B11-C08E5;

B12-K04F; C04-E03; C04-E05; C04-F09; C11-C08E3; C11-C08E5;
C12-K04F; D03-K03; D03-K04; D05-H05; D05-H12A; D05-H12D1; D05-H18B;
J04-B01;

CHEMICAL-CODES:

Chemical Indexing M1 *01*

Fragmentation Code

M423 M710 M781 M905 N102 P831 Q233 Q435 Q505

Specific Compounds

A00NSD A00NSN

Chemical Indexing M1 *02*

Fragmentation Code

M423 M710 M781 M905 N102 P831 Q233 Q435 Q505

Specific Compounds

A012PD A012PN

Chemical Indexing M1 *03*

Fragmentation Code

M417 M423 M750 M905 N102 N132 Q233 Q435

Specific Compounds

A00GTK A00GTA

Chemical Indexing M1 *04*

Fragmentation Code

M423 M750 M905 N102 N134 Q233 Q435

Specific Compounds

A00NSK A00NSA

Chemical Indexing M6 *05*

Fragmentation Code

M905 P831 Q233 Q435 Q505 R515 R521 R624 R627 R639

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: C2003-038220

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Draw	Desc	Image							

KWC

Generate Collection

Print

Term	Documents
PCR	50478
PCRS	1491
POLYMERASE	51950
POLYMERASES	7738
CHAIN	663570
CHAINS	183498
REACTION	1102160
REACTIONS	309761
(2 AND ((POLYMERASE ADJ CHAIN ADJ REACTION) OR PCR)).USPT,PGPB,EPAB,DWPI.	23
(L2 AND (PCR OR POLYMERASE CHAIN REACTION)).USPT,PGPB,EPAB,DWPI.	23

Display Format:

-

Change Format

[Previous Page](#)[Next Page](#)

(FILE 'HOME' ENTERED AT 15:46:15 ON 11 JUN 2003)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 15:46:25 ON 11 JUN 2003

L1	682 S CRUZ-PEREZ P?/AU OR BUTTNER M?/AU
L2	599 S STACHYBRTRYs OR STACHYBOTRYs CHARTARUM
L3	2040 S STACHYBOTRYs OR STACHYBOTRYs CHARTARUM
L4	96 S L3 AND (PCR OR POLYMERASE CHAIN REACTION)
L5	10 S L1 AND L3
L6	39 S L4 AND QUANTITAT?
L7	13 S L6 AND SPOR##
L8	8 DUP REM L7 (5 DUPLICATES REMOVED)
L9	13 S L6 AND REAL TIME
L10	5 DUP REM L5 (5 DUPLICATES REMOVED)
L11	17 DUP REM L6 (22 DUPLICATES REMOVED)

=>

L8 ANSWER 1 OF 8 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 2003-09056 BIOTECHDS

TITLE: Detecting and quantifying fungus **Stachybotrys chartarum** comprises isolating DNA from sample, subjecting DNA to **polymerase chain reaction** amplification using primer, and detecting fungus by visualizing amplified product;
DNA primer and DNA probe for microorganism detection and quantification

AUTHOR: CRUZ-PEREZ P; BUTTNER M P

PATENT ASSIGNEE: UNIV NEVADA LAS VEGAS

PATENT INFO: WO 2002079513 10 Oct 2002

APPLICATION INFO: WO 2002-US6335 28 Feb 2002

PRIORITY INFO: US 2001-280712 29 Mar 2001; US 2001-280712 29 Mar 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-148258 [14]

AN 2003-09056 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Detecting and quantifying the presence of fungus **Stachybotrys chartarum**, comprises isolating DNA from a sample suspected of containing the fungus *S. chartarum*, subjecting the DNA to **polymerase chain reaction** (PCR) amplification utilizing at least one primer base sequence, and detecting the fungus *S. chartarum* by visualizing the product of the PCR.

DETAILED DESCRIPTION - Detecting and quantifying the presence of fungus **Stachybotrys chartarum**, comprises isolating DNA from a sample suspected of containing the fungus *S. chartarum*, subjecting the DNA to **polymerase chain reaction** (PCR) amplification utilizing at least one primer base sequence which comprises at least one of the sequences (S1) 5'-GTTGCTTCGGCGGGAAC-3', and (S2) 5'-TTTGCGTTTGCCACTCAGAG-3', or (S3) 5'-ACCTATCGTTGCTTCGGCG-3', and (S4) 5'-GCGTTTGCCACTCAGAGAATACT-3'. Also

a

probe that is specific for the fungal species *S. chartarum*, collecting the sample from the environment, extracting the sample's DNA, obtaining DNA standards from a culture of *S. chartarum*, determining the concentration of *S. chartarum* spores in the DNA standards, amplifying by PCR each of the DNA standards and the collected sample's DNA using the obtained primer set and probe, and comparing amplification plots obtained by PCR of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *S. chartarum* in the collected sample and a concentration of the fungus *S. chartarum* in the collected sample, or detecting the fungus *S. chartarum* by visualizing the product of the PCR. INDEPENDENT CLAIMS are included for; (1) A primer set (I) for detecting *S. chartarum* using PCR, comprises a first primer comprising S1 and a second primer comprising S2, or a first primer comprising S3 and a second primer comprising S4; and (2) A primer and probe set for detecting *S. chartarum* using PCR, comprises (I) and probe comprising a base sequence 6-FAM-5'-CTGCGCCCGGATCCAGGC-3'-TAMRA.

BIOTECHNOLOGY - Preferred Method: Subjecting the DNA to PCR further uses a probe comprising a base sequence 6-FAM-5'-CTGCGCCCGGATCCAGGC-3'-TAMRA. The sample is obtained from the environment.

Preferred Primer: The first primer comprises a forward primer, and the

second primer comprises a reverse primer.

USE - The method is useful for detecting and quantifying the fungus **Stachybotrys chartarum** in a sample (claimed).

ADVANTAGE - The method detects and absolutely quantifies *S. chartarum* without the necessity of further employing estimated quantization techniques and thus avoids inaccuracies of estimated quantization, where PCR inhibitors may co-extract with the DNA.

EXAMPLE - Detecting the fungus **Stachybotrys chartarum** in a sample was performed as follows. Pure cultures of *S. chartarum* were sampled by gently swabbing the surface of the fungal colony with a cotton swab and resuspending in 3 ml poly(butylene terephthalate) (PBT). After vortexing on maximum speed for 1 minute, the swab was removed. Aliquots of 500 microl were placed in 2 ml microcentrifuge tubes for subsequent DNA extraction. Samples and aliquots

were stored at -70 degreesC. Extraction was performed using the boiling method. The boiling protocol consisted of treating 10-500 microl of the suspension with sodium dodecylsulfate 0.5% (v/v) final concentration and proteinase K (20 microg/ml final concentration), followed by incubation at 50 degreesC for 10 minutes and boiling for 15 minutes. The samples were chilled on ice for 2 minutes, and bovine serum albumin was added to a final concentration of 0.05% (w/v). The samples were incubated for 5 minutes at 37 degreesC in a rotary shaker at a speed of 225 rpm. The DNA was maintained at 4 degreesC for immediate purification by pellet paint purification. The purified DNA was subjected to **polymerase chain reaction (PCR)** amplification using the designed primers and probe for *S. chartarum*. The Primer Express software was used to generate primers and probes for the internal transcribed spacer (ITS1) of the 18S rRNA sequence of *S. chartarum* (GenBank

accession no.AF081468). Primer set had the sequences 5'-GTTGCTTCGGCGGGAAC-3' and 5'-TTTGCGTTTGCCACTCAGAG-3' for the forward (STAF1) and reverse (STAR1) primers, respectively. The fluorescent probe used for both primer sets had the sequence 6-FAM-5'-CTGCGCCCGGATCCAGGC-3'-TAMRA. An ethidium bromide dot quantization method was utilized for the determination of

the presence of DNA on samples prepared for specificity testing. Four microl of control or sample DNA was combined with an equal volume of ethidium bromide and mixed by vortexing. Negative controls were prepared by substituting TE buffer for DNA. Mixed samples were applied in the form

of a dot onto a piece of plastic wrap stretched over the surface of an UV transilluminator. quantization using 7700 sodium dodecyl sulfate (SDS) was accomplished by the use of standards of known concentration, processed in the same manner as the unknown samples. Standards (100-105 template/reaction) were amplified in duplicate with replicate unknown samples. The primer set produced 107-bp amplicon. The primer set

designed for *S. chartarum* amplified control DNA from *S. chartarum* ATCC strain 9182. *S. chartarum* primers amplified two ATCC and 17 *S. chartarum* laboratory isolates. They did not amplify fungal DNA extracted from 21 other fungal species (comprising 16 fungal genera), including three non-chartarum **Stachybotrys** species and two *Memnoniella* species. All fungal extracts tested for the presence of DNA with the dot quantization method produced positive results. Thus the results indicated

that the detection and absolute quantization of *S. chartarum* was obtained

using **quantitative PCR**. (40 pages)

L8 ANSWER 2 OF 8 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 2003:40133 SCISEARCH

THE GENUINE ARTICLE: 626LU

TITLE: Species-specific detection of three human-pathogenic microsporidial species from the genus *Encephalitozoon* via fluorogenic 5' nuclease **PCR** assays

AUTHOR: Hester J D; Varma M; Bobst A M; Ware M W; Lindquist H D A (Reprint); Schaefer F W

CORPORATE SOURCE: US EPA, Natl Exposure Res Lab, Cincinnati, OH 45268 USA (Reprint); Univ Cincinnati, Dept Chem, Cincinnati, OH 45221 USA

COUNTRY OF AUTHOR: USA

SOURCE: MOLECULAR AND CELLULAR PROBES, (DEC 2002) Vol. 16, No. 6, pp. 435-444.
Publisher: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0890-8508.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 41

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This study describes fluorogenic 5' nuclease **PCR** assays suitable for rapid, sensitive, high-throughput detection of the human-pathogenic microsporidial species *Encephalitozoon hellem*, *E. cuniculi* and *E. intestinalis*. The assays utilize species-specific primer sets and a genus-specific dual fluorescent-labeled probe that anneals to

a region within the *Encephalitozoon* 16S rRNA gene. The assay design theoretically permits the probe to be used either with one set of primers for species-level determination or with a combination of all three primer sets for a genus-level screening of samples. The linear range of all

three species-specific calibration curves that were developed using serial ten-fold dilutions of genomic DNA isolated from hemacytometer counted **spores** was determined to be between 10^4 and 10^{-1} **spores** per **PCR** sample. The coefficients of variation were less than or equal to 5.2% over the entire 5-log span of each calibration curve. When DNA isolated from flow cytometric enumerated **spores** from each of the three *Encephalitozoon* species was used to evaluate the **quantitative** capability of the species' respective calibration curves, the results from 34 out of 36 (94%) samples were within 2 standard deviations. The species-specificity of each assay was confirmed using DNA isolated from 10^4 **spores** from each of the other two *Encephalitozoon* species as well as DNA extracted from numerous other protozoa, algae and bacteria. (C) 2002 Elsevier Science Ltd. All rights reserved.

L8 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:383054 CAPLUS

DOCUMENT NUMBER: 138:33766

TITLE: Evaluation of rapid DNA extraction methods for the **quantitative** detection of fungi using real-time **PCR** analysis

AUTHOR(S): Haugland, Richard A.; Brinkman, Nichole; Vesper, Stephen J.

CORPORATE SOURCE: National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, 45268, USA

SOURCE: Journal of Microbiological Methods (2002), 50(3),
319-323
CODEN: JMIMDQ; ISSN: 0167-7012
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Three comparatively rapid methods for the extn. of DNA from fungal
conidia
and yeast cells in environmental (air, water and dust) samples were
evaluated for use in real-time PCR (TaqMan.RTM.) analyses. A
simple bead milling method was developed to provide sensitive, accurate
and precise quantification of target organisms in air and water (tap and
surface) samples. However, **quant.** anal. of dust samples
required further purifn. of the extd. DNA by a streamlined silica
adsorption procedure.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L8 ANSWER 4 OF 8 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001258684 MEDLINE
DOCUMENT NUMBER: 21141010 PubMed ID: 11246797
TITLE: Quantification of **Stachybotrys chartarum**
conidia in indoor dust using real time, fluorescent
probe-based detection of PCR products.
AUTHOR: Roe J D; Haugland R A; Vesper S J; Wymer L J
CORPORATE SOURCE: US Army Center for Health Promotion and Preventative
Medicine, Aberdeen Proving Ground, Maryland, USA.
SOURCE: JOURNAL OF EXPOSURE ANALYSIS AND ENVIRONMENTAL
EPIDEMIOLOGY, (2001 Jan-Feb) 11 (1) 12-20.
Journal code: 9111438. ISSN: 1053-4245.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517
AB Analyses of fungal **spores** or conidia in indoor dust samples can
be useful for determining the contamination status of building interiors
and in signaling instances where potentially harmful exposures of
building
occupants to these organisms may exist. A recently developed method for
the quantification of **Stachybotrys chartarum** conidia,
using real-time, fluorescence probe--based detection of PCR
products (TaqMan system) was employed to analyze indoor dust samples for
this toxigenic fungal species. Dust samples of up to 10 mg were found to
be amenable to DNA extraction and analysis. **Quantitative**
estimates of *S. chartarum* conidia in composite dust samples, containing a
four-log range of these cells, were within 25 -- 104% of the expected
quantities in 95% of analyses performed by the method. Calibrator
samples
containing known numbers of *S. chartarum* conidia were used as standards
for quantification. Conidia of an arbitrarily selected strain of
Geotrichum candidum were added in equal numbers to both dust and
calibrator samples before DNA extraction. Partial corrections for
reductions in overall DNA yields from the dust samples compared to the

calibrator samples were obtained by comparative analyses of rDNA sequence yields from these reference conidia in the two types of samples. Dust samples from two contaminated homes were determined to contain greater than 10(3) *S. chartarum* conidia per milligram in collection areas near the sites of contamination and greater than 10(2) conidia per milligram in several areas removed from these sites in analyses performed by the method. These measurements were within the predicted range of agreement with results obtained by direct microscopic enumeration of presumptive *Stachybotrys* conidia in the same samples.

L8 ANSWER 5 OF 8 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000203887 MEDLINE
DOCUMENT NUMBER: 20203887 PubMed ID: 10741843
TITLE: Evaluation of *Stachybotrys chartarum* in the house of an infant with pulmonary hemorrhage: quantitative assessment before, during, and after remediation.
AUTHOR: Vesper S; Dearborn D G; Yike I; Allan T; Sobolewski J; Hinkley S F; Jarvis B B; Haugland R A
CORPORATE SOURCE: US Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH 45268, USA.. Vesper.Stephen@EPA.gov
SOURCE: JOURNAL OF URBAN HEALTH, (2000 Mar) 77 (1) 68-85. Journal code: 9809909. ISSN: 1099-3460.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000420

AB *Stachybotrys chartarum* is an indoor mold that has been associated with pulmonary hemorrhage cases in the Cleveland, Ohio, area. This study applied two new quantitative measurements to air samples from a home in which an infant developed PH. Quantitative polymerase chain reaction and a protein synthesis inhibition assay were used to determine the level of *S. chartarum* spores and their toxicity in air samples taken before, during, and after a remediation program was implemented to remove the fungus. Initial spore concentrations were between 0.1 and 9.3 spores/m³ of air, and the toxicity of air particulates was correspondingly low. However, the dust in the house contained between

0.4 and 2.1 x 10(3) spores/mg (as determined by hemocytometer counts). The remediation program removed all contaminated wallboard, paneling, and carpeting in the water-damaged areas of the home. In addition, a sodium hypochlorite solution was used to spray all surfaces during remediation. Although spore counts and toxicity were high during remediation, air samples taken postremediation showed no detectable levels of *S. chartarum* or related toxicity. Nine isolates of *S. chartarum* obtained from the home were analyzed for spore toxicity, hemolytic activity, and random amplified polymorphic DNA banding patterns. None of the isolates produced highly toxic spores (>90 microg T2 toxin equivalents per gram wet weight spores) after growth for 10 and 30 days on wet wallboard, but three isolates were hemolytic consistently. DNA banding patterns suggested that at least one

of these isolates was related to isolates from homes of infants with previously investigated cases.

L8 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:627542 CAPLUS

DOCUMENT NUMBER: 132:45489

TITLE: **Quantitative** measurement of **Stachybotrys chartarum** conidia using real time detection of **PCR** products with the TaqManTM fluorogenic probe system

AUTHOR(S): Haugland, R. A.; Vesper, S. J.; Wymer, L. J.

CORPORATE SOURCE: National Exposure Research Laboratory, US Environmental Protection Agency, Cincinnati, OH, 45268, USA

SOURCE: Molecular and Cellular Probes (1999), 13(5), 329-340
CODEN: MCPRE6; ISSN: 0890-8508

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The occurrence of **Stachybotrys chartarum** in indoor environments has been assocd. with a no. of human health concerns, including fatal pulmonary hemosiderosis in infants. Currently used culture-based and microscopic methods of fungal species identification are

poorly suited to providing quick and accurate ests. of airborne human exposures to the toxin contg. conidia of this organism. In this study, real-time **polymerase chain reaction** (**PCR**) product anal. using the TaqMan fluorogenic probe system and an Applied Biosystems Prism model 7700 sequence detection instrument (model 7700) was applied to the specific detection of *S. chartarum* ribosomal DNA (rDNA) sequences. Based upon this assay and a recently reported comparative cycle threshold method for quantifying target DNA sequences using data from the model 7700, a simple method for the direct quantification of *S. chartarum* conidia was developed. In analyses of samples contg. several different strains and from two to over 2.times.10⁵ cells, this method consistently provided **quant.** ests. of *S. chartarum* conidia that were within a one-fold range (50-200%) of those detd. on the basis of direct microscopic counts in a hemocytometer. The method showed a similar level of agreement with direct counting in the quantification of *S. chartarum* conidia in air samples collected from several contaminated homes. (c) 1999 Academic Press.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS

FORMAT RECORD. ALL CITATIONS AVAILABLE IN THE RE

L8 ANSWER 7 OF 8 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1999:594978 SCISEARCH

THE GENUINE ARTICLE: 220VB

TITLE: Evaluation of different methods for the extraction of DNA from fungal conidia by **quantitative** competitive **PCR** analysis

AUTHOR: Haugland R A (Reprint); Heckman J L; Wymer L J

CORPORATE SOURCE: US EPA, NATL EXPOSURE RES LAB, CINCINNATI, OH 45268 (Reprint); IT CORP, TEST & EVALUAT FACIL, CINCINNATI, OH 45204

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (AUG 1999) Vol. 37, No. 2, pp. 165-176.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE
AMSTERDAM, NETHERLANDS.
ISSN: 0167-7012.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Five different DNA extraction methods were evaluated for their effectiveness in recovering **PCR** templates from the conidia of a series of fungal species often encountered in indoor air. The test organisms were *Aspergillus versicolor*, *Penicillium chrysogenum*, *Stachybotrys chartarum*, *Cladosporium herbarum* and *Alternaria alternata*. The extraction methods differed in their use of different cell lysis procedures. These included grinding in liquid nitrogen, grinding at ambient temperature, sonication, glass bead milling and freeze-thawing. DNA purification and recovery from the lysates were performed using a commercially available system based on the selective binding of nucleic acids to glass milk. A simple **quantitative competitive polymerase chain reaction (QC-PCR)** assay was developed for use in determining copy numbers of the internal transcribed spacer (ITS) regions of the ribosomal RNA operon (rDNA) in the total DNA extracts. These **quantitative** analyses demonstrated that the method using glass bead milling was most effective in recovering **PCR** templates from each of the different types of conidia both in terms of absolute copy numbers recovered and also in terms of lowest extract to extract variability. Calculations of average template copy yield per conidium in this study indicate that the bead milling method is sufficient to support the detection of less than ten conidia of each of the different organisms in a **PCR** assay. (C) 1999 Elsevier Science B.V. All rights reserved.

L8 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:284232 BIOSIS
DOCUMENT NUMBER: PREV199799583435
TITLE: **Quantitative** evaluation of different methods for extracting **PCR** templates from fungal conidia.
AUTHOR(S): Heckman, J. L.; Haugland, R. A.
CORPORATE SOURCE: USEPA, Cincinnati, OH USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1997) Vol. 97, No. 0, pp. 461.
Meeting Info.: 97th General Meeting of the American Society for Microbiology Miami Beach, Florida, USA May 4-8, 1997
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

=>

21251216 PubMed ID: 11352593
TITLE: Specific detection of **Stachybotrys chartarum** in pure culture using quantitative polymerase chain reaction.
AUTHOR: Cruz-Perez P; Buttner M P; Stetzenbach L D
CORPORATE SOURCE: Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas, 4505 S. Maryland Parkway, Las Vegas, NV 89154-4009, USA.
SOURCE: MOLECULAR AND CELLULAR PROBES, (2001 Jun) 15 (3) 129-38. Journal code: 8709751. ISSN: 0890-8508.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010820
Last Updated on STN: 20010820
Entered Medline: 20010816

AB Research was conducted with laboratory cultures to establish a protocol for the rapid detection and quantitation of the toxigenic fungus **Stachybotrys chartarum** by means of polymerase chain reaction (PCR). Sequences for the 18 S rRNA gene of *S. chartarum* were obtained from GenBank and compared against all other available sequences on-line with the Basic Local Alignment Search Tool (BLAST). Two sets of TaqMan primers and one fluorescently labelled probe were designed and tested for selectivity, specificity and sensitivity of detection. A fluorogenic nuclease assay in conjunction with a sequence detector were used for the amplification and quantitation of *S. chartarum*. The primers designed amplified all *S. chartarum* isolates tested and did not amplify DNA extracted from other **Stachybotrys** species or 15 other fungal genera. The primer set selected had a sensitivity of <23 template

copies.

Many *S. chartarum* samples were initially negative after PCR amplification.

Incorporation of an internal positive control in the PCR reaction demonstrated the presence of inhibitors in these samples. PCR inhibitors were removed by dilution or further purification of the DNA samples. The results of this research report on a quantitative PCR (QPCR) method for detection and quantitation of *S. chartarum* and demonstrate the presence

of

PCR inhibitors in some *S. chartarum* isolates.
Copyright 2001 Academic Press.

Specific detection of **Stachybotrys**

chartarum in pure culture using quantitative polymerase chain reaction.

AUTHOR(S): Cruz, P. (1); **Buttner, M. P. (1)**; Stetzenbach, L. D. (1)

CORPORATE SOURCE: (1) University of Nevada-Las Vegas, Las Vegas, NV USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 612.
<http://www.asmsusa.org/mtgsrc/generalmeeting.htm>. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.

DOCUMENT TYPE: Conference

LANGUAGE: English

AB Research was conducted with laboratory cultures to establish a protocol for the rapid detection and quantitation of the toxigenic fungus **Stachybotrys chartarum** by means of quantitative polymerase chain reaction (QPCR). Two sets of TaqMan(R) primers and one fluorescently labeled probe were designed and tested for selectivity, specificity and sensitivity of detection. A fluorogenic nuclease assay in conjunction with a sequence detector were used for the amplification and quantitation of *S. chartarum*. The primers designed amplified all *S. chartarum* isolates tested and did not amplify DNA extracted from other **Stachybotrys** species or from 15 other fungal genera. The primer set selected had a sensitivity of <23 template copies per PCR reaction. However, many *S. chartarum* samples were initially negative after PCR amplification. Incorporation of an internal positive control in the PCR reaction demonstrated the presence of inhibitors in these samples. PCR inhibitors were removed by dilution or further purification of the DNA samples. The results of this research detail a QPCR method for detection and quantitation of *S. chartarum* and demonstrate the presence of PCR inhibitors in some *S. chartarum* isolates.

A TaqMan-based PCR method for the
quantitative detection of **Stachybotrys**
chartarum conidia.

AUTHOR(S): Haugland, R. A. (1); Vesper, S. J. (1)

CORPORATE SOURCE: (1) National Exposure Research Laboratory, U.S.
Environmental Protection Agency, Cincinnati, OH USA

SOURCE: Abstracts of the General Meeting of the American Society
for Microbiology, (1999) Vol. 99, pp. 573.
Meeting Info.: 99th General Meeting of the American
Society
for Microbiology Chicago, Illinois, USA May 30-June 3,
1999
American Society for Microbiology
. ISSN: 1060-2011.

DOCUMENT TYPE: Conference

LANGUAGE: English

Evaluation of different methods for the extraction of DNA
from fungal conidia by **quantitative** competitive
PCR analysis.

AUTHOR: Haugland R A; Heckman J L; Wymer L J
CORPORATE SOURCE: National Exposure Research Laboratory, U.S. Environmental
Protection Agency, Cincinnati, OH 45268, USA..
haugland.rich@epa.gov

SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (1999 Aug) 37 (2)
165-76.
Journal code: 8306883. ISSN: 0167-7012.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990914

AB Five different DNA extraction methods were evaluated for their
effectiveness in recovering **PCR** templates from the conidia of a
series of fungal species often encountered in indoor air. The test
organisms were *Aspergillus versicolor*, *Penicillium chrysogenum*,
Stachybotrys chartarum, *Cladosporium herbarum* and
Alternaria alternata. The extraction methods differed in their use of
different cell lysis procedures. These included grinding in liquid
nitrogen, grinding at ambient temperature, sonication, glass bead milling
and freeze-thawing. DNA purification and recovery from the lysates were
performed using a commercially available system based on the selective
binding of nucleic acids to glass milk. A simple **quantitative**
competitive **polymerase chain reaction** (QC-
PCR) assay was developed for use in determining copy numbers of
the internal transcribed spacer (ITS) regions of the ribosomal RNA operon
(rDNA) in the total DNA extracts. These **quantitative** analyses
demonstrated that the method using glass bead milling was most effective
in recovering **PCR** templates from each of the different types of
conidia both in terms of absolute copy numbers recovered and also in
terms of lowest extract to extract variability. Calculations of average
template copy yield per conidium in this study indicate that the bead
milling method is sufficient to support the detection of less than ten
conidia of each of the different organisms in a **PCR** assay.

Quantification of **Stachybotrys chartarum**

conidia in indoor dust using real time, fluorescent probe-based detection of **PCR** products.
AUTHOR: Roe J D; Haugland R A; Vesper S J; Wymer L J
CORPORATE SOURCE: US Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, Maryland, USA.
SOURCE: JOURNAL OF EXPOSURE ANALYSIS AND ENVIRONMENTAL EPIDEMIOLOGY, (2001 Jan-Feb) 11 (1) 12-20.
Journal code: 9111438. ISSN: 1053-4245.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

AB Analyses of fungal spores or conidia in indoor dust samples can be useful for determining the contamination status of building interiors and in signaling instances where potentially harmful exposures of building occupants to these organisms may exist. A recently developed method for the quantification of **Stachybotrys chartarum** conidia, using real-time, fluorescence probe--based detection of **PCR** products (TaqMan system) was employed to analyze indoor dust samples for this toxigenic fungal species. Dust samples of up to 10 mg were found to be amenable to DNA extraction and analysis. **Quantitative** estimates of *S. chartarum* conidia in composite dust samples, containing a four-log range of these cells, were within 25 -- 104% of the expected quantities in 95% of analyses performed by the method. Calibrator samples containing known numbers of *S. chartarum* conidia were used as standards for quantification. Conidia of an arbitrarily selected strain of *Geotrichum candidum* were added in equal numbers to both dust and calibrator samples before DNA extraction. Partial corrections for reductions in overall DNA yields from the dust samples compared to the calibrator samples were obtained by comparative analyses of rDNA sequence yields from these reference conidia in the two types of samples. Dust samples from two contaminated homes were determined to contain greater than 10(3) *S. chartarum* conidia per milligram in collection areas near the sites of contamination and greater than 10(2) conidia per milligram in several areas removed from these sites in analyses performed by the method. These measurements were within the predicted range of agreement with results obtained by direct microscopic enumeration of presumptive **Stachybotrys** conidia in the same samples.